

DOCKET NO.: ISIS-4723  
 Application No.: 09/823,031  
 Office Action D ted: July 15, 2003

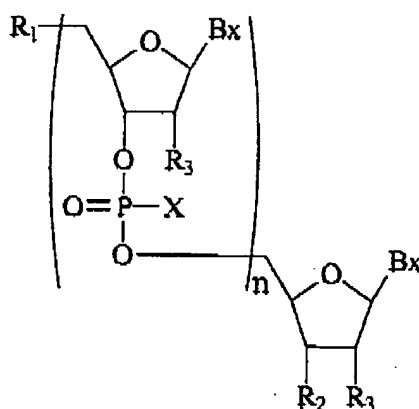
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 PROCEDURE PURSUANT TO  
 37 CFR § 1.116

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims:**

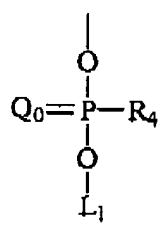
1-21. (canceled)

22. (currently amended) A process for preparing an oligonucleotide having the formula:



wherein:

R<sub>1</sub> is a group having the formula:



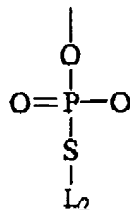
Q<sub>0</sub> is O or S;

R<sub>4</sub> is O<sup>-</sup>, hydroxyl, or a protected hydroxyl;

R<sub>2</sub> is hydroxyl, a protected hydroxyl or a group having the formula:

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each  $R_3$  is H, a 2'-substituent group or a protected 2'-substituent group;

each X is, independently, O', hydroxyl, protected hydroxyl, or -S-L<sub>3</sub>;

each Bx is an optionally protected heterocyclic base moiety;

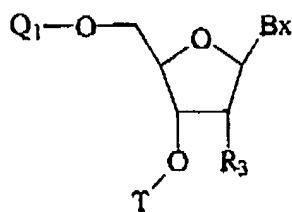
n is from 3 to about 50; and

L<sub>1</sub>, L<sub>2</sub> and each of said L<sub>3</sub> are, independently, a conjugate group cholesterol, phospholipid, biotin, phenazine, phenanthridine, anthraquinone, acridine, fluorescein, rhodamine, coumarin, or dye;

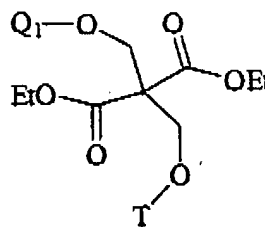
wherein said R<sub>1</sub> and at least one of said R<sub>2</sub> or said X comprise a conjugate group cholesterol, phospholipid, biotin, phenazine, phenanthridine, anthraquinone, acridine, fluorescein, rhodamine, coumarin, or dye;

comprising the steps of:

- a) providing a derivatized solid support for oligonucleotide synthesis, said derivatized solid support being derivatized with a group having one of the structures:



or



wherein

T is a bifunctional linking moiety linked to the solid support; and

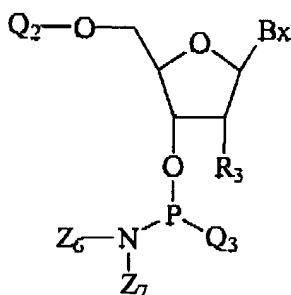
Q<sub>1</sub> is an acid labile hydroxyl protecting group;

- b) treating said derivatized solid support with an acidic reagent to deblock said acid labile hydroxyl protecting group to give a free hydroxyl group;

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c) reacting said free hydroxyl group with a phosphoramidite composition to form an extended compound, said phosphoramidite composition having the formula:



wherein

Q<sub>2</sub> is a 5'-terminal acid labile hydroxyl protecting group;

Q<sub>3</sub> is a phosphorus protecting group; and

Z<sub>6</sub> and Z<sub>7</sub> are, independently, C<sub>1-6</sub> alkyl;

or Z<sub>6</sub> and Z<sub>7</sub> are joined together to form a 4- to 7-membered heterocyclic ring system including the nitrogen atom to which Z<sub>6</sub> and Z<sub>7</sub> are attached, wherein said ring system optionally includes at least one additional heteroatom selected from O, N and S;

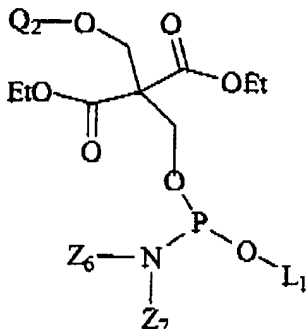
d) oxidizing said extended compound to form an oxidized compound, or treating said extended compound with an acidic reagent to deblock said 5'-terminal acid labile hydroxyl protecting group of said extended compound to give a free hydroxyl group and repeating step c) at least one time followed by oxidizing said extended compound to form an oxidized compound;

e) treating said oxidized compound with an acidic reagent to deblock said acid labile hydroxyl protecting group to give a free hydroxyl group and repeating steps c) and d) at least three times to form an extended oxidized compound;

f) treating said extended oxidized compound with a reagent effective to deblock said protected hydroxyl group to give a free hydroxyl group and reacting said free hydroxyl group with a compound of formula:

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thereby forming a 5'-functionalized compound;

wherein

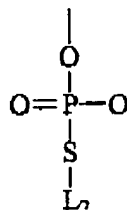
Q<sub>5</sub> is an acid labile hydroxyl protecting group;

g) treating said 5'-functionalized compound for a time and under conditions effective to remove at least one phosphorus protecting group giving at least one deblocked phosphorothioate linkage; and

h) reacting said deblocked phosphorothioate linkage with a conjugate group cholesterol, phospholipid, biotin, phenazine, phenanthridine, anthraquinone, acridine, fluorescein, rhodamine, coumarin, or dye that is reactive with and forms a covalent bond with said deblocked phosphorothioate linkage to give said oligonucleotide.

23. (original) The process of Claim 22 further comprising the step of treating said 5'-functionalized compound with a capping agent to form a capped compound.

24. (original) The process of Claim 22 wherein said R<sub>2</sub> is a group having the formula:



25. (original) The process of Claim 24 wherein L<sub>1</sub> is different from L<sub>2</sub>.

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26. (original) The process of Claim 22 wherein at least one of said X is -S-L<sub>3</sub>.
27. (original) The process of Claim 26 wherein L<sub>1</sub> is different from L<sub>3</sub>.
28. (canceled)
29. (canceled)
30. (currently amended) The process of ~~Claim 1~~ Claim 22 wherein each of said Q<sub>3</sub> is independently selected from the group consisting of cyanoethyl, diphenylsilylethyl, cyanobutenyl, cyano *p*-xylyl (CPX), methyl-N-trifluoroacetyl ethyl (META) and acetoxy phenoxy ethyl (APOE) groups.
31. (original) The process of Claim 22 wherein said 5'-functionalized compound is treated in step g) to remove all phosphorus protecting groups.
32. (original) The process of Claim 22 wherein n is from about 8 to about 30.
33. (original) The process of Claim 32 wherein n is from about 15 to about 25.
34. (original) The process of Claim 22 wherein each of said Q<sub>1</sub> and Q<sub>2</sub> is independently selected from the group consisting of trimethoxytrityl, dimethoxytrityl (DMT), monomethoxytrityl, 9-phenylxanthen-9-yl (Pixyl) and 9-(*p*-methoxyphenyl)xanthen-9-yl (Mox).
35. (original) The process of Claim 22 wherein each of said Bx is independently selected from the group consisting of adenine, guanine, thymine, cytosine, uracil, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine, 2-thiocytosine, 5-halouracil, 5-halocytosine, 5-propynyl uracil, 5-propynyl cytosine, 6-azo uracil, 6-azo cytosine, 6-azo

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thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-substituted adenines and guanines, 5-substituted uracils and cytosines, 7-methylguanine, 7-methyladenine, 8-azaguanine, 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine and 3-deazaadenine.

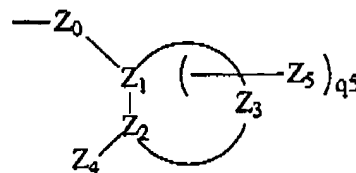
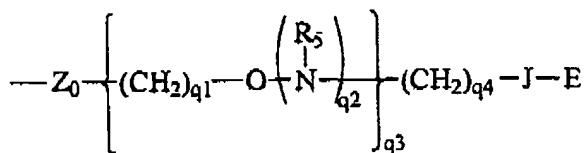
36. (original) The process of Claim 22 wherein at least one of said L<sub>1</sub>, L<sub>2</sub>, and L<sub>3</sub> is attached to the oligonucleotide through a linking group.

37. (original) The process of Claim 36 wherein the linking group comprises a dialkylglycerol linker.

38. (original) The process of Claim 22 wherein each of said  $Z_6$  and  $Z_7$  is isopropyl.

39. (original) The process of Claim 22 wherein each R<sub>3</sub> is, independently, C<sub>1</sub>-C<sub>20</sub> alkyl, C<sub>2</sub>-C<sub>20</sub> alkenyl, C<sub>2</sub>-C<sub>20</sub> alkynyl, C<sub>5</sub>-C<sub>20</sub> aryl, O-alkyl, O-alkenyl, O-alkynyl, O-alkylamino, O-alkylalkoxy, O-alkylaminoalkyl, O-alkyl imidazole, thiol, S-alkyl, S-alkenyl, S-alkynyl, NH-alkyl, NH-alkenyl, NH-alkynyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, N-phthalimido, halogen keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, heterocycle, carbocycle, polyamine, polyamide, polyalkylene glycol, and polyether;

or each substituent group has one of formula I or II:



# I

## II

wherein:

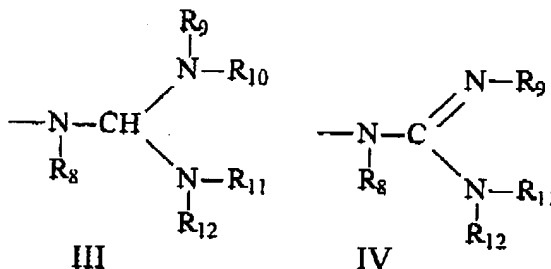
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$Z_0$  is O, S or NH;

J is a single bond, O or C(=O);

E is  $C_1$ - $C_{10}$  alkyl,  $N(R_5)(R_6)$ ,  $N(R_5)(R_7)$ ,  $N=C(R_5)(R_6)$ ,  $N=C(R_5)(R_7)$  or has one of formula III or IV;



each  $R_8$ ,  $R_9$ ,  $R_{10}$ ,  $R_{11}$  and  $R_{12}$  is, independently, hydrogen,  $C(O)R_{13}$ , substituted or unsubstituted  $C_1$ - $C_{10}$  alkyl, substituted or unsubstituted  $C_2$ - $C_{10}$  alkenyl, substituted or unsubstituted  $C_2$ - $C_{10}$  alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or optionally,  $R_9$  and  $R_{10}$ , together form a phthalimido moiety with the nitrogen atom to which they are attached;

or optionally,  $R_{11}$  and  $R_{12}$ , together form a phthalimido moiety with the nitrogen atom to which they are attached;

each  $R_{13}$  is, independently, substituted or unsubstituted  $C_1$ - $C_{10}$  alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

$R_5$  is T-L,

T is a bond or a linking moiety;

L is a chemical functional group, a conjugate group or a solid support material;

each  $R_5$  and  $R_6$  is, independently, H, a nitrogen protecting group, substituted or unsubstituted  $C_1$ - $C_{10}$  alkyl, substituted or unsubstituted  $C_2$ - $C_{10}$  alkenyl, substituted or unsubstituted  $C_2$ - $C_{10}$  alkynyl, wherein said substitution is  $OR_3$ ,  $SR_3$ ,  $NH_3^+$ ,  $N(R_{14})(R_{15})$ , guanidino or acyl where said acyl is an acid amide or an ester;

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or  $R_5$  and  $R_6$ , together, are a nitrogen protecting group or are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

or  $R_{21}$ , T and L, together, are a chemical functional group;

each  $R_{14}$  and  $R_{15}$  is, independently, H,  $C_1$ - $C_{10}$  alkyl, a nitrogen protecting group, or  $R_{14}$  and  $R_{15}$ , together, are a nitrogen protecting group;

or  $R_{14}$  and  $R_{15}$  are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

$Z_4$  is OX, SX, or  $N(X)_2$ ;

each X is, independently, H,  $C_1$ - $C_8$  alkyl,  $C_1$ - $C_8$  haloalkyl,  $C(=NH)N(H)R_{16}$ ,  $C(=O)N(H)R_{16}$  or  $OC(=O)N(H)R_{16}$ ;

$R_{16}$  is H or  $C_1$ - $C_8$  alkyl;

$Z_1$ ,  $Z_2$  and  $Z_3$  comprise a ring system having from about 4 to about 7 carbon atoms or having from about 3 to about 6 carbon atoms and 1 or 2 heteroatoms wherein said heteroatoms are selected from oxygen, nitrogen and sulfur and wherein said ring system is aliphatic, unsaturated aliphatic, aromatic, or saturated or unsaturated heterocyclic;

$Z_5$  is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about 14 carbon atoms,  $N(R_5)(R_6)OR_5$ , halo,  $SR_5$  or CN;

each  $q_1$  is, independently, an integer from 1 to 10;

each  $q_2$  is, independently, 0 or 1;

$q_3$  is 0 or an integer from 1 to 10;

$q_4$  is an integer from 1 to 10;

$q_5$  is from 0, 1 or 2; and

provided that when  $q_3$  is 0,  $q_4$  is greater than 1.

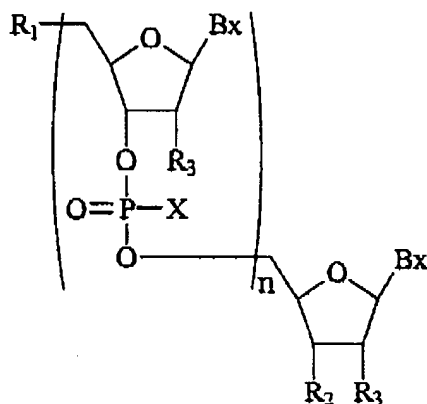
40-49. (canceled)

50. (currently amended) A process for preparing an oligonucleotide having the formula:



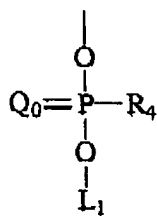
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wherein:

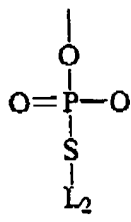
R<sub>1</sub> is a group having the formula:



Q<sub>0</sub> is O or S;

R<sub>4</sub> is O<sup>-</sup>, hydroxyl, or a protected hydroxyl;

R<sub>2</sub> is hydroxyl, a protected hydroxyl or a group having the formula:



each R<sub>3</sub> is H, a 2'-substituent group or a protected 2'-substituent group;

each X is, independently, O<sup>-</sup>, hydroxyl, a protected hydroxyl, or -S-L<sub>3</sub>;

each Bx is an optionally protected heterocyclic base moiety;

n is from 3 to about 50; and

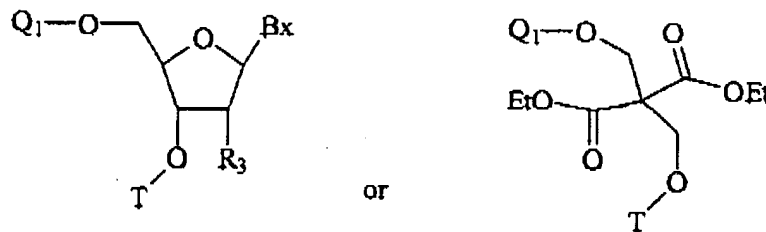
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$L_1$ ,  $L_2$  and each of said  $L_3$  are, independently, a ~~conjugate group~~ cholesterol, phospholipid, biotin, phenazine, phenanthridine, anthraquinone, acridine, fluorescein, rhodamine, coumarin, or dye;

comprising the steps of:

- a) providing a derivatized solid support for oligonucleotide synthesis, said derivatized solid support being derivatized with a group having one of the structures:



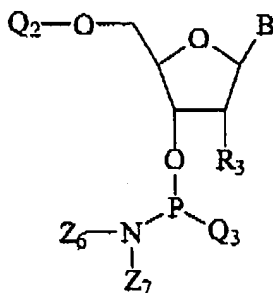
wherein

T is a bifunctional linking moiety linked to the solid support; and

$Q_1$  is an acid labile hydroxyl protecting group;

- b) treating said derivatized solid support with an acidic reagent to deblock said acid labile hydroxyl protecting group to give a free hydroxyl group;

- c) reacting said free hydroxyl group with a phosphoramidite composition to form an extended compound, said phosphoramidite composition having the formula:



wherein

$Q_2$  is a 5'-terminal acid labile hydroxyl protecting group;

$Q_3$  is a phosphorus protecting group; and

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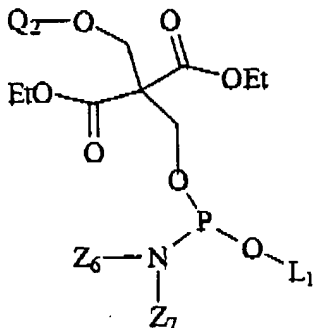
$Z_6$  and  $Z_7$  are, independently,  $C_{1-6}$  alkyl;

or  $Z_6$  and  $Z_7$  are joined together to form a 4- to 7-membered heterocyclic ring system including the nitrogen atom to which  $Z_6$  and  $Z_7$  are attached, wherein said ring system optionally includes at least one additional heteroatom selected from O, N and S;

d) oxidizing said extended compound to form an oxidized compound, or treating said extended compound with an acidic reagent to deblock said 5'-terminal acid labile hydroxyl protecting group of said extended compound to give a free hydroxyl group and repeating step c) at least one time followed by oxidizing said extended compound to form an oxidized compound;

e) treating said oxidized compound with an acidic reagent to deblock said acid labile hydroxyl protecting group to give a free hydroxyl group and repeating steps c) and d) at least three times to form an extended oxidized compound;

f) treating said extended oxidized compound with an acidic reagent effective to deblock said 5'-terminal acid labile hydroxyl protecting group to give a free hydroxyl group and reacting said free hydroxyl group with a compound of the formula:



thereby forming a 5'-functionalized compound;

wherein

$Q_5$  is an acid labile hydroxyl protecting group;

51. (original) The process of Claim 50 further comprising the step of treating said 5'-functionalized compound with a capping agent to form a capped compound.

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52. (original) The process of Claim 50 wherein at least one of said  $L_1$ ,  $L_2$ , and  $L_3$  is attached to the oligonucleotide through a linking group.

53. (original) The process of Claim 52 wherein the linking group comprises a dialkylglycerol linker.

54. (original) The process of Claim 50 wherein each of said  $Z_6$  and  $Z_7$  is isopropyl.

55. (canceled)

56. (canceled)

57. (original) The process of Claim 50 wherein  $L_1$  is different from  $L_2$  and  $L_3$ .

58. (original) The process of Claim 50 wherein each of said  $Q_3$  is independently selected from the group consisting of cyanoethyl, diphenylsilylethyl, cyanobutenyl, cyano *p*-xylyl (CPX), methyl-N-trifluoroacetyl ethyl (META) and acetoxy phenoxy ethyl (APOE) groups.

59. (original) The process of Claim 50 wherein each of said  $Q_1$  and  $Q_2$  is independently selected from the group consisting of trimethoxytrityl, dimethoxytrityl (DMT), monomethoxytrityl, 9-phenylxanthen-9-yl (Pixyl) and 9-(*p*-methoxyphenyl)xanthen-9-yl (Mox).

60. (original) The process of Claim 50 wherein each of said  $B_x$  is independently selected from the group consisting of adenine, guanine, thymine, cytosine, uracil, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine, 2-thiocytosine, 5-halouracil, 5-halocytosine, 5-propynyl uracil, 5-propynyl cytosine, 6-azo uracil, 6-azo cytosine, 6-azo thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-substituted adenines and guanines, 5-

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CI  
substituted uracils and cytosines, 7-methylguanine, 7-methyladenine, 8-azaguanine, 8-  
azaadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine and 3-deazaadenine.

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